Distribution and geochemical behaviour of anionic surfactants determined as ethyl violet active substances in Lake Biwa, Japan

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Abstract

The distribution and geochemical behavior of anionic surfactants (AS) determined as ethyl violet active substances (EVAS) were studied in Lake Biwa, Japan. We determined the vertical distributions of dissolved EVAS (DEVAS), suspended particulate EVAS (PEVAS), particulate organic carbon (POC), and the PEVAS/POC ratio. Statistical analyses conducted using plots of PEVAS vs. POC and PEVAS/ Chlorophyll-*a* (Chl-*a*) vs. POC/Chl-*a* revealed that the distributions of DEVAS and PEVAS were affected by the concentrations of freshly produced POC and aged POC and by the pH of the lake water. Adsorption and desorption of EVAS onto the POC phase were found to be important mechanisms regulating the distribution and environmental/geochemical behavior of EVAS in the lake. The organic carbon-normalized particle–water partitioning coefficient, K_{OC}, for EVAS was also estimated and compared to that for nonylphenol.

Keywords

anionic surfactants; ethyl violet; distribution, geochemical behavior, Lake Biwa

Introduction

Linear alkylbenzene sulfonate (LAS) is the primary anionic surfactant found in Japan. This compound is listed as Class I in the Pollutant Release and Transfer Register Law of Japan. A Class I Designated Chemical Substance is defined as continuously occurring throughout much of the environment based on its physical and chemical properties, manufactured volume, and importation and usage, and having risks to human health and the ecosystem (Japan Ministry of the Environment 2013). Production of LAS was 72 kt in 2002 and the amount of LAS discharged to the sewage in Japan was 64 kt in 2001 (National Institute of Technology and Evaluation 2005). The degree of discharge is related to the environmental risk.

Research on anionic surfactants (AS) in the aquatic environment has been carried out globally. In Japan, AS concentrations have been reported to range

between 50–8500 μg/L in river water (Kobayashii and Yamauchi 1979; Kobuke 1981; Yoshimura *et al*. 1984; Hirayama *et al*. 2006), 0–264 μg/g in river sediments (Ambe 1972; Uchiyama 1982; Yoshimura *et al*. 1984), and 10–50 μg/g in lake sediments (Ambe 1972). LAS concentrations ranged between 0.6–79 μg/L in river water in Japan (Kobuke 2004; Miura *et al*. 2008), 0–342 μg/L in South Africa (Gordon *et al*. 2009), and 2–126 μg/L in the Philippines (Dyer *et al*. 2003). LAS concentrations also ranged between 60–2300 μg/g in suspended particles in rivers in Japan (Takada and Ishiwatari 1987) and between 0.03–17.76 μg/g and 0.101–0.186 μg/g in river sediments in Portugal (Hampel *et al*. 2009) and South Africa (Gordon *et al*. 2009), respectively.

As noted above, most limnological and environmental studies of AS have been focused on rivers, but few studies have been conducted of AS in lakes. The distribution of AS in rivers is important in determining anthropogenic pollution levels and discharges to the natural environment of AS by human activities. However, these studies alone are not sufficient to fully delineate the

environmental and geochemical processes of AS, because water masses are constantly renewed by flows from upstream. On the other hand, water masses may stagnate in lakes and thus, studies on partitioning of AS between lake water and suspended and sediment particles are more feasible.

Several authors have studied removal processes for AS in laboratory experiments (Ou *et al*. 1996; Perales *et al*. 1999; Doi *et al*. 2002; Garcia *et al*. 2002). These compounds are generally known to be degraded by microorganisms (Perales *et al*. 1999) and adsorbed by solid phases (Ou *et al*. 1996; Doi *et al*. 2002; Garcia *et al*. 2002). The former depends on the water temperature, while the latter is related to the organic carbon content of the solid phase, known as the particulate organic carbon (POC) content. To date, there have been few studies of adsorption and partitioning of AS to POC in lakes. Although some authors have addressed POC, they treated it as a homogeneous material despite being a mixture of various organic materials (Yoshida *et al*. 2009). In our previous study (Yoshida *et al*. 2009), we clarified that POC in lakes

could be classified into two fractions, freshly produced POC (f-POC) and aged POC (a-POC), based on differences in the affinity of nonylphenol (NP) to POC; its affinity to a-POC was approximately 4 times higher than that to f-POC. A similar evaluation is also needed for AS.

Colorimetric methods have often been used for analysis of AS. Among these methods, methylene blue, which offers the advantage of being an easy-to-use and convenient technique, is widely used. In recent years, the methylene blue reagent has been replaced by an ethyl violet reagent (Soejima 1982). The ethyl violet colorimetric method is more useful than methylene blue as it has: (1) high sensitivity, (2) a very simple and less time-consuming procedure, (3) little interference by coexisting ions, and (4) good reproducibility and quantification of trace amounts of anionic surfactants (Motomizu *et al*. 1982). It is also used as a standard method in Japan (Japan Standards Association 1998). Therefore, we quantified AS as ethyl violet active substances (EVAS).

In this study, we determined the distribution and geochemical behavior of AS in

Lake Biwa as EVAS. Using plots of suspended particulate EVAS (PEVAS) vs. POC and PEVAS/Chlorophyll-*a* (Chl-*a*) vs. POC/Chl-*a*, we evaluated adsorption and partitioning of EVAS to POC in Lake Biwa. The average PEVAS/POC ratio and organic carbon-normalized particle–water partitioning coefficient (K_{OC}) of EVAS to f-POC and a-POC were estimated and compared to those of NP.

Materials and methods

Solvents and standards

Sodium sulfate and sodium hydroxide were purchased from Nacalai Tesque (Kyoto, Japan). Acetic acid, toluene, disodium ethylenediaminetetraacetate (EDTA), and sodium dodecyl sulfate (SDS) were obtained from Wako Chemicals (Osaka, Japan). Ethyl violet was obtained from Tokyo Chemical Industry (Tokyo, Japan).

Sampling site and sample collection

The sampling sites for this study were located in Lake Biwa (35°15' N, 136°05' E) and the Yasu River. Lake Biwa, which is the largest lake in Japan, is divided into two parts, the northern and southern basins. The former has a volume of 27.3 km^3 with an average depth of 44 m. Thermal stratification typically occurs from May to January and vertical convection of the entire water column in the lake occurs regularly from February to April. The southern basin of the lake has a volume of 0.2 km^3 with an average depth of 3.5 m. The water in this basin is not thermally stratified and is more or less saturated with atmospheric oxygen throughout the year (Sugiyama *et al*. 2005). Field surveys were conducted at Station Ie-1 (35°12.58' N, 135°59.55' E) between September 2000 and August 2001.

The Yasu River is a large river that flows near Station Ie-1 in Lake Biwa. It has the largest drainage area and the second highest water discharge among the more than 110 rivers flowing into Lake Biwa (Lake Biwa Environmental Research Institute 1986; Kunimatsu 1988). Several towns are located along this

118 river with a total population of 220,000. The river water is generally used for
119 industrial water, domestic supply, and irrigation, and then the discharge is
120 released to the river (Kimaro *et al*. 2002). Field surveys of the river were
121 conducted at Stations YS-1 (35°09.70' N, 135°99.30' E) and YS-2 (35°02.37' N,
122 136°01.12' E) on 5 September 2000 and 9 March 2001.

123 Lake water samples were collected vertically with a Van Dorn water sampler
124 and river water samples were collected with a glass bottle. The samples were
125 filtered through a Whatman GF/F filter (diameter 47 mm, pore size 0.7 μm) to
126 separate suspended particles from the water. The water samples were analyzed
127 within 3 days of sampling. Suspended particle samples collected on the filter
128 were kept frozen until they were analyzed.

129

130 **Analytical procedures**

131 Sample treatment for AS determination was carried out as follows. Forty
132 milliliters of a water sample was transferred into a glass centrifuge tube. Two

133 milliliters 1 mol/L sodium sulfate solution, 2 mL acetic acid–EDTA buffer (pH 5.0),
134 0.8 mL 1 mmol/L ethyl violet solution, and 2 mL toluene were added to the tube.
135 The tube was shaken for 60 min and then centrifuged for 5 min. After phase
136 separation, the absorbance of the toluene phase was measured at 611 nm
137 (UV-1600; Shimadzu, Kyoto, Japan) and calibrated against an SDS solution.
138 Suspended particle and sediment samples were dried at 60 °C and
139 ultrasonicated twice with 10 mL methanol for 45 min at room temperature. The
140 obtained extracts were evaporated until they were dry using a centrifugal
141 vaporizer (Model CVE 2wd; Tokyo Rika Kikai, Tokyo, Japan). The residue was
142 redissolved in 40 mL water and the solution was then treated as a water sample.
143 POC was quantified with a CHN analyzer (Model 2400; Perkin Elmer, Tokyo,
144 Japan). The temperatures of the combustion and reduction chambers were set
145 to 925 °C and 640 °C, respectively.
146 Chl-*a* was quantified spectrophotometrically according to the methods of
147 UNESCO and Lorenzen (Lorenzen 1967).

148

149 # Results and discussion

150 ## Method validation

151 In the analyses using the ethyl violet colorimetric method, sodium sulfate was
152 used to accelerate phase separation and EDTA was used to reduce
153 interferences from multivalent metal ions. AS were determined as EVAS.
154 Method validation was carried out to evaluate the suitability of this method. SDS
155 was used as a standard material for analysis of AS. The linearity of its calibration
156 curve was evaluated by taking measurements at four different concentrations of
157 SDS. The correlation coefficient *r* of the curve was >0.999 from 0 to 38 μg/L SDS.
158 The detection limit was determined as the concentration of SDS that gave a
159 signal three times the background noise, which was 0.27 μg/L.

160 Accuracy and precision were calculated based on recovery of SDS from
161 sediments, since sediments have a more complex matrix. For this evaluation,
162 101 μg/g SDS was spiked into a sediment sample containing 235 μg/g of EVAS.

163 The recovery rate and precision were 115% and 3.3% (n = 3). Thus, this method
164 was found to be suitable for analysis of AS in environmental and geochemical
165 studies.
166
167 **Distribution and geochemical behavior of EVAS in Lake Biwa**
168 Figure 1 shows the vertical distributions for water temperature, pH, and EVAS
169 concentration in Lake Biwa. During the circulation period (2 April 2001), the
170 vertical distribution of dissolved EVAS (DEVAS) was relatively constant,
171 because the lake water was well mixed due to the vertical convection that occurs
172 in early spring. Conversely, the DEVAS concentration during the stagnation
173 period (12 September and 5 October 2000, 11 April 2001) had higher values at
174 the surface and decreased at the thermocline. The concentration in the
175 hypolimnion was nearly constant except near the bottom and was similar to that
176 during the circulation period. The high DEVAS concentrations in the epilimnion
177 are believed to be due to river water with high DEVAS concentrations flowing

178 only into the epilimnion and/or to desorption of AS from the POC phase due to
179 the increase in pH in the surface water during the stagnation period. Average
180 DEVAS concentrations in the Yasu River were 18.2 μg/L on 5 September 2000
181 and 55.7 μg/L on 9 March 2001. These values were higher than those in the
182 epilimnion during the stagnation period and in the entire water column during the
183 circulation period. Weighted average concentrations in the epilimnion were 12.2
184 μg/L on 12 September 2000, 14.6 μg/L on 5 October 2000, and 12.2 μg/L on 11
185 April 2001. During the circulation period (2 April 2001), the weighted average
186 concentration over the entire water column was 10.5 μg/L. These results indicate
187 that the DEVAS concentration in the surface water is increased by the Yasu and
188 other rivers flowing into the epilimnion during the stagnation period.

189 In addition, the pH in the epilimnion increases with active biological
190 productivity in the summer in many lakes, including Lake Biwa (Lake Biwa
191 Research Institute and National Institute for Research Advancement 1984;
192 Sugiyama *et al*. 2005). It has been reported that AS desorb from the suspended

193 POC phase when the pH of the aqueous phase increases (Yoshimura and
194 Nakae 1982). Therefore, the DEVAS concentration in the surface water may
195 have increased by desorption of AS from the POC phase in the suspended
196 particulate matter and the sediments in shallow and littoral area of the lake when
197 the pH of the water increased. This hypothesis was supported by the results for
198 PEVAS vs. POC (see below).

199 The concentration of PEVAS decreased from the surface to the thermocline or
200 the middle layer. However, at any given sampling time in Fig. 1, the vertical
201 distribution of PEVAS/POC, which represents the organic carbon-normalized
202 concentration of EVAS in the suspended particles, was neither uniform nor
203 similar to those of PEVAS or POC. Figure 2 plots PEVAS vs. POC. The
204 correlation coefficient *r* was relatively low at 0.619. There are believed to be two
205 reasons for this result. The first is the treatment of POC as a homogeneous
206 material despite actually being a mixture of various organic materials. The
207 second is not considering changes in the pH of the lake water, although AS is

known to desorb from the suspended POC phase when the pH of the aqueous phase increases.

The first issue can be explained further based on the results of our previous environmental and geochemical study on NP in Lake Biwa (Yoshida *et al*. 2009). In that study, POC was mathematically classified into two fractions, f-POC and a-POC, based on the affinity of NP to these fractions. The differences between f-POC and a-POC are likely associated with their chemical structure and the nature of the organic matter. Therefore, in the present study, we examined the distribution of PEVAS and POC in a similar manner. The total concentration of POC, [POC], can be expressed as:

$$[POC] = [POC]_f + [POC]_a$$

where $[POC]_f$ and $[POC]_a$ are the concentrations of f-POC and a-POC, respectively. If the EVAS concentration accumulated by f-POC and a-POC are defined as $[PEVAS]_f$, and $[PEVAS]_a$, respectively, then the total PEVAS concentration, [PEVAS], can be expressed as:

223 $$[PEVAS] = [PEVAS]_f + [PEVAS]_a$$

224 $$= R_f[POC]_f + R_a[POC]_a$$

225 $$= R_f[POC]_f + R_a([POC] - [POC]_f)$$

226 where R_f and R_a are the average PEVAS/POC ratios for f-POC and a-POC,

227 respectively.

228 Phytoplankton is typical of freshly produced organic matter in a lake and the

229 Chl-*a* concentration is an index of phytoplankton abundance. Therefore, if Chl-*a*

230 is regarded as an index of the f-POC concentration, $[POC]_f$ can be expressed as:

231 $$[POC]_f = \alpha[\text{Chl-}a]$$

232 where α is the average f-POC/Chl-*a* ratio. The following relationship can be

233 obtained from the above equations:

234 $$[PEVAS] = R_f\alpha[\text{Chl-}a] + R_a([POC] - \alpha[\text{Chl-}a])$$

235 $$= R_a[POC] + \alpha(R_f - R_a)[\text{Chl-}a]$$

236 $$[PEVAS]/[\text{Chl-}a] = R_a[POC]/[\text{Chl-}a] + \alpha(R_f - R_a)$$

237 The obtained equation can be interpreted as follows. The plot of PEVAS/Chl-*a*

238 vs. POC/Chl-*a* is predicted to be linear, with a slope and intercept of R_a and $\alpha(R_f$
239 $- R_a)$, respectively. If AS accumulates in a-POC more than in f-POC (i.e., $R_a >$
240 $R_f)$, the intercept will be negative. Figure 3 plots PEVAS/Chl-*a* vs. POC/Chl-*a*.
241 The plot showed good linearity and its correlation coefficient (r = 0.916) was
242 higher than that of the PEVAS vs. POC plot (r = 0.619). These results suggest
243 that classification of POC into f-POC and a-POC is possible through this method
244 and is important for understanding the behavior of AS in the lake. As expected,
245 the intercept $\alpha(R_f - R_a)$ of the plot was negative (-1.27×10^{-2} g/g). Therefore, the
246 affinity of AS to a-POC was higher than to f-POC. These results are consistent
247 with our previous study showing that NP also has stronger affinity to a-POC than
248 to f-POC (Yoshida *et al*. 2009).

249 The slope R_a and intercept $\alpha(R_f - R_a)$ of the linear regression line in Fig. 3 were
250 5.5×10^{-3} g/g and -1.27×10^{-2} g/g, respectively. Using 87.8 g/g as the average α
251 value (Yoshida *et al*. 2009) based on the average POC/Chl-*a* value in culture
252 experiments of phytoplankton from Lake Biwa (Tezuka 1985), R_f can be

253 estimated as 5.4×10^{-3} g/g. According to the definition given above, R_f and R_a are
254 the average EVAS/POC ratios for f-POC and a-POC, respectively. Therefore,
255 these values indicate that the affinity of AS to a-POC is somewhat higher than
256 that to f-POC.

257 The second reason for the poor correlation coefficient (r = 0.619) between
258 PEVAS and POC in Fig. 2 can be explained by the PEVAS vs. POC plots shown
259 in Fig. 4. Based on the pH of the lake water, the data can be classified into 3
260 groups: Group A, samples of the epilimnion in September and October 2000 (pH
261 7.6–7.8; 0–13 m in September and 0–17 m in October); Group B, samples of the
262 hypolimnion in September and October 2000 (pH 6.9–7.1; 20–72 m); and Group
263 C, samples at all depths on 2 and 11 April 2001 (pH 7.1–7.4). The correlation
264 coefficients for the three groups were 0.690, 0.926, and 0.908, respectively. The
265 PEVAS/POC value (3.38) for the epilimnion with high pH values was smaller
266 than those (5.57 and 7.84) for the hypolimnion and at all depths with low pH
267 values. These results are consistent with the increase in the DEVAS

concentration in the epilimnion with high pH values during the stagnation period, and also indicates that PEVAS concentrations in the POC decreased with an increase in the pH of the lake water. In other words, EVAS is desorbed from the suspended POC phase when the pH of the aqueous phase increases.

We previously reported that some oxyanions are also released from solid phases such as sediments and suspended particulate matter with an increase in the pH of lake water (Harita *et al*. 2005), similar to the present results. Therefore, adsorption and desorption of chemical components onto solid phases with changes in the pH of lake water are considered to be important mechanisms regulating their distribution and environmental/geochemical behavior in lakes. However, such a trend was not observed in our previous study of NP. This is likely because NP, as a non-ionic compound, is not affected by changes in pH.

As shown in Fig. 4, the PEVAS/POC value (5.57) in the hypolimnion in September and October was smaller than that (7.84) at all depths in April. This is believed to be due to degradation of PEVAS in the hypolimnion during the

283 stagnation period.

284

285 **Comparison of K_{oc} values for EVAS and NP**

286 The partition coefficient K_{oc} is calculated using the following equation, which

287 uses the DEVAS concentration and the PEVAS/POC ratio (R_f and R_a):

288 $$K_{oc}(\text{mL/g – POC}) = [\text{PEVAS/POC(g/g – POC)}]/[\text{DEVAS(g/mL)}]$$

289 The average DEVAS concentrations were 15.9×10^{-9} g/mL and 9.1×10^{-9} g/mL in

290 the epilimnion and hypolimnion, respectively. Using these concentrations, log

291 K_{oc} was determined to be 5.5 and 5.8 for R_f and R_a, respectively. These values

292 are within those (2.5–5.9) obtained in a previous study (Vincent and Glenwood

293 1987).

294 The log K_{oc} values for EVAS (5.5–5.8) were higher than those for NP (4.8 and

295 5.4). The high K_{oc} values for EVAS are likely due to formation of a bulky ionic

296 pair with cationic organic compounds such as quaternary ammonium ions as

297 well as its high affinity to POC. Cationic surfactants, which typically contain

298 quaternary ammonium ions, are widely used in industrial and household
299 detergents and are discharged into lake and river waters. Their production in
300 Japan was 70 kt in 1997 (Japan Surfactant Industry Association 1997). Cationic
301 surfactants easily form a stable ion pair with coexisting anionic surfactants in
302 environmental waters (Utsunomiya *et al*. 1998) and this ion pair is quickly
303 absorbed onto sewage sludge and sediments with a high partitioning coefficient
304 (Sun *et al*. 2003; Sütterlin *et al*. 2008).

305

306 ## Conclusions

307 We studied the distribution and geochemical behavior of EVAS in Lake Biwa,
308 Japan. The distributions of DEVAS and PEVAS were clearly affected by the
309 concentrations of the two POC fractions, f-POC and a-POC, and by the pH of the
310 lake water. Thus, adsorption and desorption of EVAS onto the POC phase were
311 found to be important mechanisms regulating their distribution and
312 environmental/geochemical behavior in lakes.

313

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322 References

323 Ambe, Y. (1972) Distribution of Alkylbenzenesulfonate in the Bottom Muds of
324 Lake Suwa. *Jpn. J. Limnol.*, **33**, 44–50 (in Japanese).

325 Doi, J., Marks, K. H., DeCarvalho, A. J., McAvoy, D. C., Nielsen, A. M., Kravetz,
326 L. and Cano, M. L. (2002) Investigation of an Onsite Wastewater Treatment
327 System in Sandy Soil: Sorption and Biodegradation of Linear Alkylbenzene

328 Sulfonate. *Environ. Toxicol. Chem.*, **21** (12), 2617–2622.

329 Dyer, S. D., Peng, C., McAvoy, D. C., Fendinger, N. J., Masscheleyn, P., Castillo,
330 L. V. and Lim, J. M. (2003) The Influence of Untreated Wastewater to
331 Aquatic Communities in the Balatuin River, The Philippines. *Chemosphere*,
332 **52** (1), 43–53.

333 Garcia, M. T., Campos, E., Dalmau, M., Ribosa, I. and Sánchez-Leal, J. (2002)
334 Structure-activity Relationships for Association of Linear Alkylbenzene
335 Sulfonates with Activated Sludge. *Chemosphere*, **49** (3), 279–286.

336 Gordon, A. K., Muller, W. J., Gysman, N., Marshall, S. J., Sparham, C. J.,
337 O'Connor, S. M. and Whelan, M. J. (2009) Effect of Laundry Activities on
338 In-stream Concentrations of Linear Alkylbenzene Sulfonate in a Small Rural
339 South African River. *Sci. Total. Environ*., **407** (15), 4465–4471.

340 Hampel, M., Canário, J., Branco, V., Vale, C. and Blasco, J. (2009)
341 Environmental Levels of Linear Alkylbenzene Sulfonates (LAS) in Sediments
342 from the Tagus Estuary (Portugal): Environmental Implications. *Environ.*

343 *Monit. Assess*., **149** (1–4), 151–161.

344 Harita, Y., Hori, T. and Sugiyama, M. (2005) Release of Trace Oxyanions from
345 Littoral Sediments and Suspended Particles Induced by pH Increase in the
346 Epilimnion of Lakes. *Limnol. Oceanogr*., **50** (2), 636–645.

347 Hirayama, Y., Ikegami, H., Machida, M. and Tatsumoto, H. (2006) Simple and
348 Rapid Determination of Linear Alkylbenzene Sulfonates by In-tube
349 Solid-phase Microextraction Coupled with Liquid Chromatography. *J. Health.*
350 *Sci*., **52** (3), 228–236.

351 Japan Ministry of the Environment (2014) PRTR Substances.
352 http://www.env.go.jp/en/chemi/prtr/substances/index.html [accessed 23
353 January 2014]

354 Japan Standards Association. (1998) Japanese Industrial Standards K0101.
355 Japan Standards Association, Tokyo.

356 Japan Surfactant Industry Association. (1997) Annual Report of Japan. Japan
357 Surfactant Industry Association, Tokyo.

358 Kimaro, T., Tachikawa, Y. and Takara, K. (2002) Development of a Hydrological
359 Model for Predicting the Effects of Land Use Changes at Yasu River Basin.
360 *Proceedings of the International Conference on Urban Hydrology for the 21st*
361 *Century*, pp. 696–705.
362 Kobayashi, S. and Yamauchi, K. (1979) LAS (MBAS) and Phosphate in Nagara
363 River Water. *Jpn. J. Limnol.*, **40** (1), 29–39 (in Japanese).
364 Kobuke, Y. (1981) Anionic Surfactant Concentration in the River and Sea Waters
365 of Hyogo Prefecture and its Relationship to Phosphorus Concentration. *Jpn.*
366 *J. Limnol*., **42** (4), 189–200 (in Japanese).
367 Kobuke, Y. (2004) LAS in Urban Rivers and Factors Contributing to Reduction of
368 their Concentrations. *Water. Sci. Technol.*, **50** (5), 355–361.
369 Kunimatsu, T. (1988) Mechanism of Pollution Discharged from River and
370 Presumption of Pollution Load into Lake Biwa . *Biwako Kenkyusyo*
371 *Go-syunen Kinenshi*, 49–63 (in Japanese).
372 Lake Biwa Research Institute and National Institute for Research Advancement.

(1984) Data Book of World Lakes. 513 LECS '84, Otsu, Japan.

Lake Biwa Environmental Research Institute. (1986) Data Book of Shiga Prefecture Environmental Atlas. Lake Biwa Environmental Research Institute, Shiga, Japan.

Lorenzen, C. J. (1967) Determination of Chlorophyll and Pheopigments, Spectrophotometric Equations. *Limnol. Oceanogr.*, **12** (2), 343–346.

Miura, K., Nishiyama, N. and Yamamoto, A. (2008) Aquatic Environmental Monitoring of Detergent Surfactants. *J. Oleo. Sci.*, **57** (3), 161–170.

Motomlzu, S., Fujlwara, S., Fujiwara, A. and Toei, K. (1982) Solvent Extraction-Spectrophotometric Determination of Anionic sSurfactants with Ethyl Violet. *Anal. Chem.*, **54** (3), 392–397.

National Institute of Technology and Evaluation (2005) Linear Alkylbenzene Sulfonic Acid and its Salt. *Initial Risk Assessment Report*, **1**, 4–7.

Ou, Z. A., Yediler, Y., He, Y., Jia, L., Kettrup, A. and Sun, T. (1996) Adsorption of Linear Alkylbenzene Sulfonate (LAS) on Soils. *Chemosphere*, **32** (5),

827–839.

Perales, J. A., Manzano, M. A., Sales, D. and Quiroga, J. A. (1999) Biodegradation Kinetics of LAS in River Water. *Int. Biodeterior. Biodegradation*, **43** (4), 155–160.

Soejima, K. (1996) Studies on Spectrophotometric Determination of Anionic Surfactants with Ethyl Violet. *Saga-ken Kankyo Senta Shoho*, **11**, 56–59 (in Japanese).

Sugiyama, M., Hori, T., Kihara, S. and Matsui, M. (2005) Geochemical Behavior of Trace Elements in Lake Biwa. *Limnology*, **6** (2), 117–130.

Sun, H. F., Takata, A., Hata, N., Kasahara, I. and Taguchi S (2003) Transportation and Fate of Cationic Surfactant in River Water. *J. Environ. Monit.*, **5**, 891–895.

Sütterlin, H., Alexy, R., Coker, A. and Kümmerer, K. (2008) Mixtures of Quaternary Ammonium Compounds and Anionic Organic Compounds in the Aquatic Environment: Elimination and Biodegradability in the Closed Bottle

Test Monitored by LC–MS/MS. *Chemosphere*, **72** (3), 479–484.

Takada, H. and Ishiwatari, R. (1987) Linear Alkylbenzenes in Urban Riverine Environments in Tokyo: Distribution, Source, and Behavior. *Environ. Sci. Technol.*, **21** (9), 875–883.

Tezuka, Y. (1985) C:N:P Ratios of Seston in Lake Biwa as Indicators of Nutrient Deficiency in Phytoplankton and Decomposition Process of Hypolimnetic Particulate Matter. *Jpn. J. Limnol*., **46** (4), 239–246 (in Japanese).

Uchiyama, M. (1982) The Distribution of Methylene Blue Active Substances (MBAS) in Lake Oze. *Jpn. J. Limnol*., **43** (1), 39–43 (in Japanese).

Utsunomiya, A., Mori, Y. and Hasegawa, K. (1998) Adsorption of Linear Alkylbenzenesulfonates and their Complexes with Cationic Surfactants on River Sediment, and their Biodegradation in River Water. *Jpn. J. Toxicol. Environ. Health*, **44** (4), 264–276 (in Japanese).

Vincent, C. H. and Glenwood, K. W. (1987) Structure-Activity Relationships for Sorption of Linear Alkylbenzenesulfonates. *Environ. Sci. Technol.*, **21** (4),

370–373.

Yoshida, H., Kudari, S., Hori, T. and Sugiyama, M. (2009) Distribution of Particulate Nonylphenol in Lake Biwa, Japan. *Water. Air. Soil. Pollut*., **200** (1–4), 267–276.

Yoshimura, K. and Nakae, A. (1982) Studies on the Biodegradation of Linear Alkylbenzene Sulfonate by Activated Sludge (I). J*pn. J. Water. Pollut. Res*., **5** (2), 19–25 (in Japanese).

Yoshimura, K., Hayashi, K., Kawase, J. and Tsuji, K. (1984) Existence of Anionic Surfactants in River. *Jpn. J. Limnol*., **45** (1), 51–60 (in Japanese).

Figure captions

Fig. 1. Vertical distributions of WT, pH, DEVAS, PEVAS, POC, PEVAS/POC, and Chl-*a* at Station Ie-1 in Lake Biwa. WT: water temperature.

Fig. 2. Plot of PEVAS vs. POC. The regression line for the plot of PEVAS vs. POC was constrained to pass through the origin to estimate an average PEVAS/POC ratio. The correlation coefficient *r* was estimated from the PEVAS and POC data.

Fig. 3. Plot of PEVAS/Chl-*a* vs. POC/Chl-*a*. The correlation coefficient *r* was estimated from the PEVAS/Chl-*a* and POC/Chl-*a* data.

Fig. 4. Plot of PEVAS vs. POC. The data were classified into 3 groups: Group A, samples of the epilimnion (squares) in September and October 2000 (pH

443 7.6–7.8; 0–13 m in September and 0–17 m in October); Group B, samples of the
444 hypolimnion (triangles) in September and October 2000 (pH 6.9–7.1; 20–72 m);
445 and Group C, samples from all depths (circles) on 2 and 11 April 2001 (pH
446 7.1–7.4). The regression line for the plot of PEVAS vs. POC was constrained to
447 pass through the origin to estimate an average PEVAS/POC ratio. The
448 correlation coefficient *r* was estimated from the PEVAS and POC data.
449

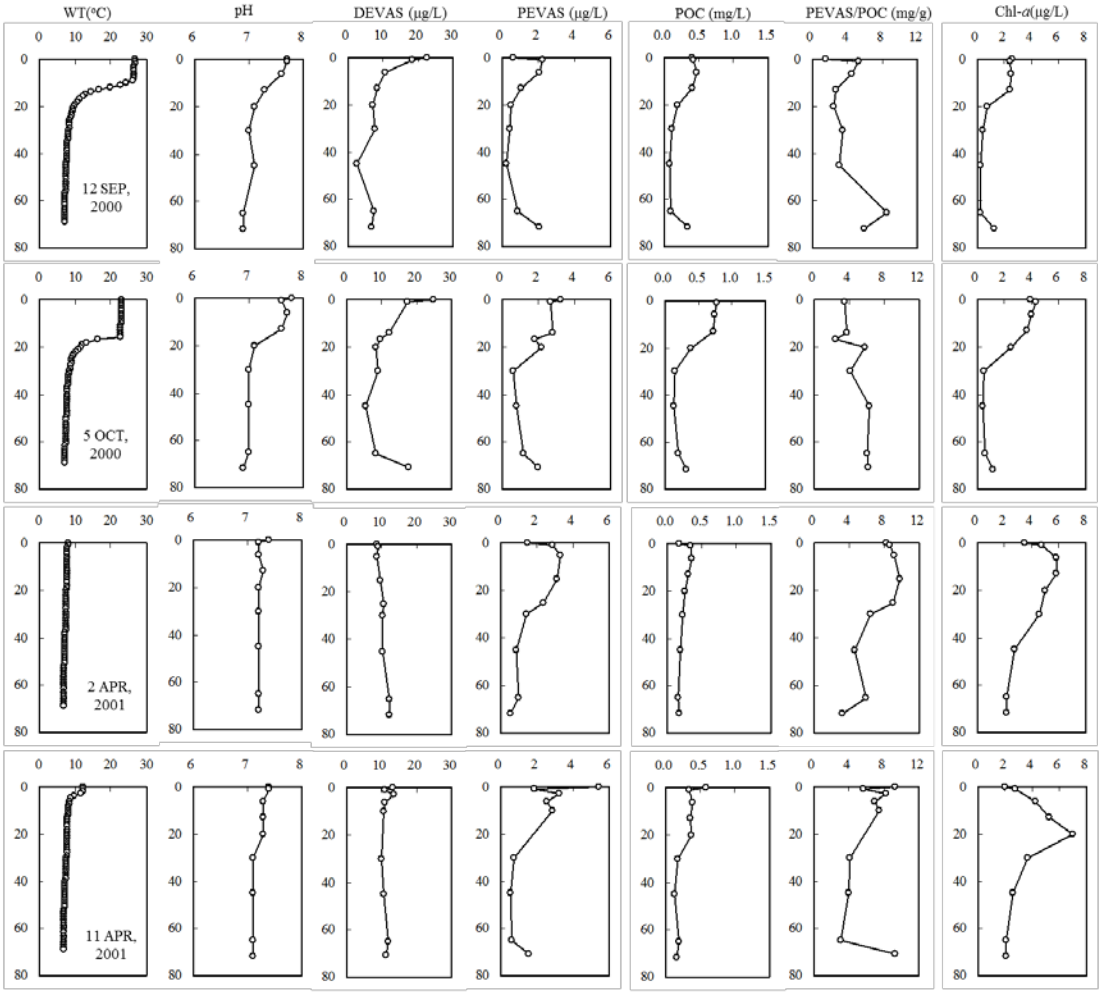
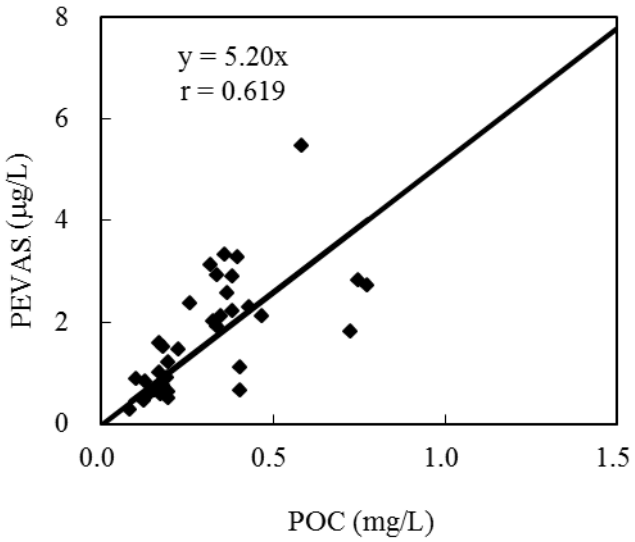


Fig. 1.



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453 **Fig. 2.**

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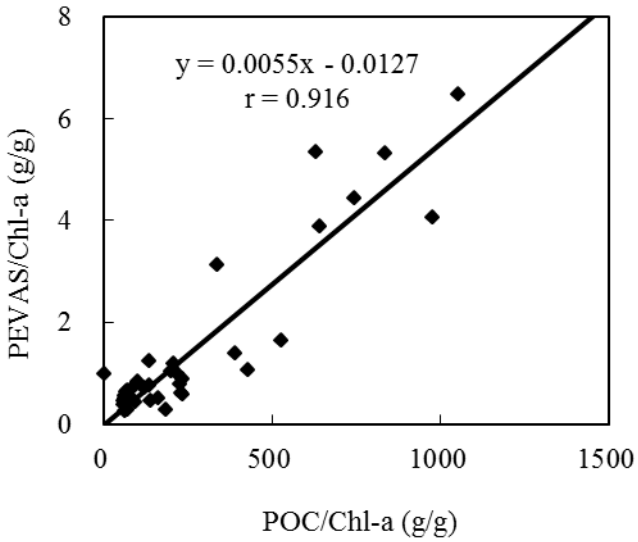

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456 **Fig. 3.**

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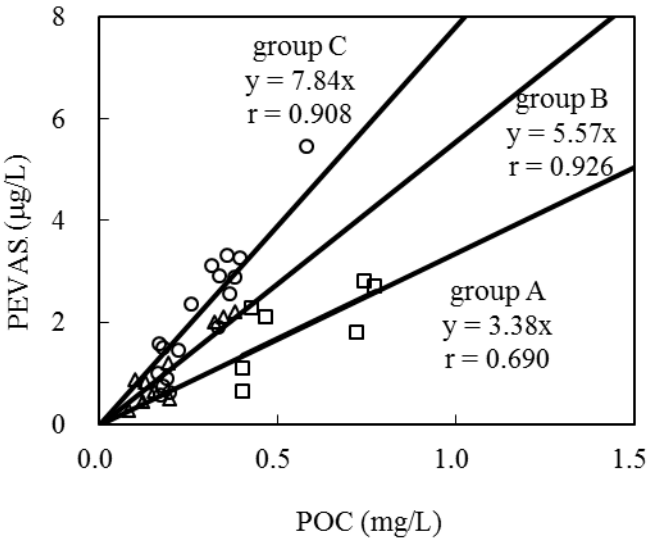

Fig. 4.